(19) Europäisch s Pat ntamt
European Patent Office
Office européen des brevets

(11) EP 0 869 179 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
07.10.1998 Bulletin 1998/41

(21) Application number: **98302528.9**

(22) Date of filing: **01.04.1998**

(51) Int Cl.[6]: **C12N 15/12**, C07K 14/715, A61K 48/00, G01N 33/50, C07K 16/28

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **02.04.1997 US 41796 P**
28.10.1997 US 959382

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(54) Tumor necrosis related receptor, TR7

(57) TR7 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR7 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others and diagnostic assays for such conditions.

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Description

This application claims the benefit of U.S. Provisional Application No. 60/041,796, filed April 2, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Tumor necrosis factor receptor (TNF-R) family, hereinafter referred to as TR7. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, such as responses to certain stimuli and natural biological processes, are controlled by factors such as cytokines. Cytokines generally act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF-ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized. Identified ligands include TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-IBBL, OX40L and nerve growth factor (NGF). The superfamily of TNF-receptors include the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, which implies that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants which abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., Science 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. et al, Cell 69:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α acting through their receptors include hemorrhagic necrosis of transplanted tumors, cytotoxicity, endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmuine disease, AIDS and graft-versus-host rejection (Beutler, B. and Von Huffel, C., Science 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an approximately 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas has been reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., Cell 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TR7 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TR7 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TR7 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TR7 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TR7" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said TR7 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TR7.

"TR7 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor forma-

are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO: 1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such TR7 polynucleotides.

TR7 of the invention is structurally related to other proteins of the Tumor necrosis factor receptor (TNF-R) family, as shown by the results of sequencing the cDNA encoding human TR7. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 7 to 1974) encoding a polypeptide of 655 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 35% identity (using BLAST (from GCG)) in 168 amino acid residues with human osteoprotegerin (OPG) protein (Simonet WS, et al., Cell 89: 309-319 (1997)). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 65% identity (using BESTFIT (from GCG)) in 102 nucleotide residues with Human Herpvesvirus Entry Mediator (HVEM) (Montgomery, R. I. et al., Cell 87, 427 (1996)) and 57% identity (using BESTFIT (from GCG)) in 118 nucleotide residues with human Osteoprotegerin (OPG) Protein (Simonet WS, et al., Cell 89: 309-319 (1997)). Thus TR7 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1[a]

```
   1  TCAGCCATGG GGACCTCTCC GAGCAGCAGC ACCGCCCTCG CCTCCTGCAG

  51  CCGCATCGCC CGCCGAGCCA CAGCCACGAT GATCGCGGGC TCCCTTCTCC

 101  TGCTTGGATT CCTTAGCACC ACCACAGCTC AGCCAGAACA GAAGGCCTCG

 151  AATCTCATTG GCACATACCG CCATGTTGAC CGTGCCACCG GCCAGGTGCT

 201  AACCTGTGAC AAGTGTCCAG CAGGAACCTA TGTCTCTGAG CATTGTACCA

 251  ACACAAGCCT GCGCGTCTGC AGCAGTTGCC CTGTGGGGAC CTTTACCAGG
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301  CATGAGAATG GCATAGAGAA ATGCCATGAC TGTAGTCAGC CATGCCCATG

351  GCCAATGATT GAGAAATTAC CTTGTGCTGC CTTGACTGAC CGAGAATGCA

401  CTTGCCCACC TGGCATGTTC CAGTCTAACG CTACCTGTGC CCCCCATACG

451  GTGTGTCCTG TGGGTTGGGG TGTGCGGAAG AAAGGGACAG AGACTGAGGA

501  TGTGCGGTGT AAGCAGTGTG CTCGGGGTAC CTTCTCAGAT GTGCCTTCTA

551  GTGTGATGAA ATGCAAAGCA TACACAGACT GTCTGAGTCA GAACCTGGTG

601  GTGATCAAGC CGGGGACCAA GGAGACAGAC AACGTCTGTG GCACACTCCC

651  GTCCTTCTCC AGCTCCACCT CACCTTCCCC TGGCACAGCC ATCTTTCCAC

701  GCCCTGAGCA CATGGAAACC CATGAAGTCC CTTCCTCCAC TTATGTTCCC

751  AAAGGCATGA ACTCAACAGA ATCCAACTCT TCTGCCTCTG TTAGACCAAA

801  GGTACTGAGT AGCATCCAGG AAGGGACAGT CCCTGACAAC ACAAGCTCAG

851  CAAGGGGGAA GGAAGACGTG AACAAGACCC TCCCAAACCT TCAGGTAGTC

901  AACCACCAGC AAGGCCCCCA CCACAGACAC ATCCTGAAGC TGCTGCCGTC

951  CATGGAGGCC ACTGGGGGCG AGAAGTCCAG CACGCCCATC AAGGGCCCCA

1001 AGAGGGGACA TCCTAGACAG AACCTACACA AGCATTTTGA CATCAATGAG

1051 CATTTGCCCT GGATGATTGT GCTTTTCCTG CTGCTGGTGC TTGTGGTGAT

1101 TGTGGTGTGC AGTATCCGGA AAAGCTCGAG GACTCTGAAA AAGGGGCCCC

1151 GGCAGGATCC CAGTGCCATT GTGGAAAAGG CAGGGCTGAA GAAATCCATG

1201 ACTCCAACCC AGAACCGGGA GAAATGGATC TACTACTGCA ATGGCCATGG

1251 TATCGATATC CTGAAGCTTG TAGCAGCCCA AGTGGGAAGC CAGTGGAAAG

1301 ATATCTATCA GTTTCTTTGC AATGCCAGTG AGAGGGAGGT TGCTGCTTTC
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1351  TCCAATGGGT ACACAGCCGA CCACGAGCGG GCCTACGCAG CTCTGCAGCA

1401  CTGGACCATC CGGGGCCCCG AGGCCAGCCT CGCCCAGCTA ATTAGCGCCC

1451  TGCGCCAGCA CCGGAGAAAC GATGTTGTGG AGAAGATTCG TGGGCTGATG

1501  GAAGACACCA CCCAGCTGGA AACTGACAAA CTAGCTCTCC CGATGAGCCC

1551  CAGCCCGCTT AGCCCGAGCC CCATCCCCAG CCCCAACGCG AAACTTGAGA

1601  ATTCCGCTCT CCTGACGGTG GAGCCTTCCC CACAGGACAA GAACAAGGGC

1651  TTCTTCGTGG ATGAGTCGGA GCCCCTTCTC CGCTGTGACT CTACATCCAG

1701  CGGCTCCTCC GCGCTGAGCA GGAACGGTTC CTTTATTACC AAAGAAAAGA

1751  AGGACACAGT GTTGCGGCAG GTACGCCTGG ACCCCTGTGA CTTGCAGCCT

1801  ATCTTTGATG ACATGCTCCA CTTTCTAAAT CCTGAGGAGC TGCGGGTGAT

1851  TGAAGAGATT CCCCAGGCTG AGGACAAACT AGACCGGCTA TTCGAAATTA

1901  TTGGAGTCAA GAGCCAGGAA GCCAGCCAGA CCCTCCTGGA CTCTGTTTAT

1951  AGCCATCTTC CTGACCTGCT GTAGAACATA GGGATACTGC ATTCTGGAAA

2001  TTACTCAATT TAGTGGCAGG GTGGTTTTTT AATTTTCTTC TGTTTCTGAT

2051  TTTTGTTGTT TGGGGTGTGT GTGTGTGTTT GTGTGTGTGT GTGTGTGTGT

2101  GTGTGTGTGT GTTTAACAGA GAAAATGGGC AGTGCTTGAA TTCTTTCTCC

2151  TTCTCTCTCT CTCTTTTTTT TTTAAATAAC TCCTCT
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A nucleotide sequence of a human TR7 (SEQ ID NO: 1).

Table 2[b]

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  1  MGTSPSSSTA LASCSRIARR ATARMIAGSL LLLGFLSTTT AQPEQKASNL

 51  IGTYRHVDRA TGQVLTCDKC PAGTYVSEHC TNTSLRVCSS CPVGTFTRHE

101  NGIEKCHDCS QPCPWPMIEK LPCAALTDRE CTCPPGMFQS NATCAPHTVC

151  PVGWGVRKKG TETEDVRCKQ CARGTFSDVP SSVMKCKAYT DCLSQNLVVI

201  KPGTKETDNV CGTLPSFSSS TSPSPGTAIF PRPEHMETHE VPSSTYVPKG

251  MNSTESNSSA SVRPKVLSSI QEGTVPDNTS SARGKEDVNK TLPNLQVVNH

301  QQGPHHRHIL KLLPSMEATG GEKSSTPIKG PKRGHPRQNL HKHFDINEHL

351  PWMIVLFLLL VLVVIVVCSI RKSSRTLKKG PRQDPSAIVE KAGLKKSMTP

401  TQNREKWIYY CNGHGIDILK LVAAQVGSQW KDIYQFLCNA SEREVAAFSN

451  GYTADHERAY AALQHWTIRG PEASLAQLIS ALRQHRRNDV VEKIRGLMED

501  TTQLETDKLA LPMSPSPLSP SPIPSPNAKL ENSALLTVEP SPQDKNKGFF

551  VDESEPLLRC DSTSSGSSAL SRNGSFITKE KKDTVLRQVR LDPCDLQPIF

601  DDMLHFLNPE ELRVIEEIPQ AEDKLDRLFE IIGVKSQEAS QTLLDSVYSH

651  LPDLL*
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[b] An amino acid sequence of a human TR7 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding TR7 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human brain, heart, lung, thymus, kidney, small intestine, prostate, monocytes and endothelial cells, using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature,* (1992) *355*:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3- 174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding TR7 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 7 to 1974 of SEQ ID NO: 1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TR7 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The

es, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL (supra).*

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TR7 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TR7 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TR7 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TR7 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TR7 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TR7. Individuals carrying mutations in the TR7 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TR7 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al., Science* (1985) 230: 1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al., Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TR7 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease through detection of mutation in the TR7 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of TR7 polypeptide or TR7 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TR7, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel

disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, which comprises:

(a) a TR7 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof ;
(b) a nucleotide sequence complementary to that of (a);
(c) a TR7 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
(d) an antibody to a TR7 polypeptide, preferably to the polypeptide of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).
The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TR7 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TR7 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al., Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TR7 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TR7 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TR7 polypeptide via a vector directing expression of TR7 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TR7 polypeptide wherein the composition comprises a TR7 polypeptide or TR7 gene. The vaccine formulation may further comprise a suitable carrier. Since TR7 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The TR7 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al., Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TR7 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate TR7 on the one hand and which can inhibit the function of TR7 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (such as inflammatory bowel disease and psoriasis), transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer (such as lymphoproliferative disorders), atherosclerosis, and Alzheimers disease.

In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor, using detection systems appropriate to the cells bearing the receptor at their surfaces. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing a TR7 polypeptide to form a mixture, measuring TR7 activity in the mixture, and comparing the TR7 activity of the mixture to a standard.

The TR7 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of TR7 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of TR7 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of TR7 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues. Standard methods for conducting screening assays are well understood in the art.

Examples of potential TR7 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the TR7, e.g., a fragment of the ligand, or small molecules which bind to the receptor but do not elicit a response, so that the activity of the receptor is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for TR7 polypeptides; or compounds which decrease or enhance the production of TR7 polypeptides, which comprises:

(a) a TR7 polypeptide, preferably that of SEQ ID NO:2;
(b) a recombinant cell expressing a TR7 polypeptide, preferably that of SEQ ID NO:2;
(c) a cell membrane expressing a TR7 polypeptide; preferably that of SEQ ID NO: 2; or
(d) antibody to a TR7 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of TR7 activity.

If the activity of TR7 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the TR7, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of TR7 polypeptides still capable of binding the ligand in competition with endogenous TR7 may be administered. Typical embodiments of such competitors comprise fragments of the TR7 polypeptide.

In still another approach, expression of the gene encoding endogenous TR7 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al., Nucleic Acids Res* (1979) 6:3073; Cooney *et al., Science* (1988) 241:456; Dervan *et al., Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TR7 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TR7, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TR7 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches,* (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

Peptides, such as the soluble form of TR7 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration ofthese compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in

view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Example 1

Three ESTs (EST#1502886,EST#843791 and EST#2051015) with sequence similarity to the human TNF receptor were discovered in a commercial EST database. Analysis of two (EST# 843791 and EST# 2051015) nucleotide sequences, revealed that each was a partial sequence of the complete coding sequence, overlapping, with 100% identity, 108 bp at the nucleotide level. Together, the two sequences encompassed the complete predicted coding sequence of 1,968 bp, and encoded an open reading frame for a novel member of the TNF receptor superfamily and named TR7. The predicted protein is 655 amino acids long with a hydrophobic membrane spanning region indicating that at least one form of TR7 is expressed as a membrane-bound protein. Comparison of TR7 protein sequence, with other TNF receptor family proteins indicates that it has four of the cysteine-rich repeats which are characteristic of the extracellular domains of this family, and of an intracellular death domain.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Annex to the description

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: Tumor Necrosis Related Receptor, TR7

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual Property
(B) STREET: Two New Horizons Court
(C) CITY: Brentford
(D) STATE: Middlesex
(E) COUNTRY: United Kingdom
(F) ZIP: TW8 9EP

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 28-OCT-1997
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/041,796
(B) FILING DATE: 02-APR-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: GIDDINGS, Peter John

(B) REGISTRATION NUMBER: 5630

(C) REFERENCE/DOCKET NUMBER: GH-50017

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +44 181 975 6331

(B) TELEFAX: +44 181 975 6294

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2186 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCAGCCATGG GGACCTCTCC GAGCAGCAGC ACCGCCCTCG CCTCCTGCAG CCGCATCGCC    60
CGCCGAGCCA CAGCCACGAT GATCGCGGGC TCCCTTCTCC TGCTTGGATT CCTTAGCACC   120
ACCACAGCTC AGCCAGAACA GAAGGCCTCG AATCTCATTG GCACATACCG CCATGTTGAC   180
CGTGCCACCG GCCAGGTGCT AACCTGTGAC AAGTGTCCAG CAGGAACCTA TGTCTCTGAG   240
CATTGTACCA ACACAAGCCT GCGCGTCTGC AGCAGTTGCC CTGTGGGGAC CTTTACCAGG   300
CATGAGAATG GCATAGAGAA ATGCCATGAC TGTAGTCAGC CATGCCCATG GCCAATGATT   360
GAGAAATTAC CTTGTGCTGC CTTGACTGAC CGAGAATGCA CTTGCCCACC TGGCATGTTC   420
CAGTCTAACG CTACCTGTGC CCCCCATACG GTGTGTCCTG TGGGTTGGGG TGTGCGGAAG   480
AAAGGGACAG AGACTGAGGA TGTGCGGTGT AAGCAGTGTG CTCGGGGTAC CTTCTCAGAT   540
GTGCCTTCTA GTGTGATGAA ATGCAAAGCA TACACAGACT GTCTGAGTCA GAACCTGGTG   600
GTGATCAAGC CGGGGACCAA GGAGACAGAC AACGTCTGTG GCACACTCCC GTCCTTCTCC   660
AGCTCCACCT CACCTTCCCC TGGCACAGCC ATCTTTCCAC GCCCTGAGCA CATGGAAACC   720
CATGAAGTCC CTTCCTCCAC TTATGTTCCC AAAGGCATGA ACTCAACAGA ATCCAACTCT   780
TCTGCCTCTG TTAGACCAAA GGTACTGAGT AGCATCCAGG AAGGGACAGT CCCTGACAAC   840
ACAAGCTCAG CAAGGGGGAA GGAAGACGTG AACAAGACCC TCCCAAACCT TCAGGTAGTC   900
AACCACCAGC AAGGCCCCCA CCACAGACAC ATCCTGAAGC TGCTGCCGTC CATGGAGGCC   960
ACTGGGGGCG AGAAGTCCAG CACGCCCATC AAGGGCCCCA AGAGGGGACA TCCTAGACAG  1020
AACCTACACA AGCATTTTGA CATCAATGAG CATTTGCCCT GGATGATTGT GCTTTTCCTG  1080
CTGCTGGTGC TTGTGGTGAT TGTGGTGTGC AGTATCCGGA AAAGCTCGAG GACTCTGAAA  1140
AAGGGGCCCC GGCAGGATCC CAGTGCCATT GTGGAAAAGG CAGGGCTGAA GAAATCCATG  1200
ACTCCAACCC AGAACCGGGA GAAATGGATC TACTACTGCA ATGGCCATGG TATCGATATC  1260
CTGAAGCTTG TAGCAGCCCA AGTGGGAAGC CAGTGGAAAG ATATCTATCA GTTTCTTTGC  1320
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AATGCCAGTG AGAGGGAGGT TGCTGCTTTC TCCAATGGGT ACACAGCCGA CCACGAGCGG   1380
GCCTACGCAG CTCTGCAGCA CTGGACCATC CGGGGCCCCG AGGCCAGCCT CGCCCAGCTA   1440
ATTAGCGCCC TGCGCCAGCA CCGGAGAAAC GATGTTGTGG AGAAGATTCG TGGGCTGATG   1500
GAAGACACCA CCCAGCTGGA AACTGACAAA CTAGCTCTCC CGATGAGCCC CAGCCCGCTT   1560
AGCCCGAGCC CCATCCCCAG CCCCAACGCG AAACTTGAGA ATTCCGCTCT CCTGACGGTG   1620
GAGCCTTCCC CACAGGACAA GAACAAGGGC TTCTTCGTGG ATGAGTCGGA GCCCCTTCTC   1680
CGCTGTGACT CTACATCCAG CGGCTCCTCC GCGCTGAGCA GGAACGGTTC CTTTATTACC   1740
AAAGAAAAGA AGGACACAGT GTTGCGGCAG GTACGCCTGG ACCCCTGTGA CTTGCAGCCT   1800
ATCTTTGATG ACATGCTCCA CTTTCTAAAT CCTGAGGAGC TGCGGGTGAT TGAAGAGATT   1860
CCCCAGGCTG AGGACAAACT AGACCGGCTA TTCGAAATTA TTGGAGTCAA GAGCCAGGAA   1920
GCCAGCCAGA CCCTCCTGGA CTCTGTTTAT AGCCATCTTC CTGACCTGCT GTAGAACATA   1980
GGGATACTGC ATTCTGGAAA TTACTCAATT TAGTGGCAGG GTGGTTTTTT AATTTTCTTC   2040
TGTTTCTGAT TTTTGTTGTT TGGGGTGTGT GTGTGTGTTT GTGTGTGTGT GTGTGTGTGT   2100
GTGTGTGTGT GTTTAACAGA GAAAATGGGC AGTGCTTGAA TTCTTTCTCC TTCTCTCTCT   2160
CTCTTTTTTT TTTAAATAAC TCCTCT                                        2186
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 655 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Gly Thr Ser Pro Ser Ser Ser Thr Ala Leu Ala Ser Cys Ser Arg
 1               5                  10                  15
Ile Ala Arg Arg Ala Thr Ala Arg Met Ile Ala Gly Ser Leu Leu Leu
            20                  25                  30
Leu Gly Phe Leu Ser Thr Thr Thr Ala Gln Pro Glu Gln Lys Ala Ser
        35                  40                  45
Asn Leu Ile Gly Thr Tyr Arg His Val Asp Arg Ala Thr Gly Gln Val
    50                  55                  60
Leu Thr Cys Asp Lys Cys Pro Ala Gly Thr Tyr Val Ser Glu His Cys
65                  70                  75                  80
Thr Asn Thr Ser Leu Arg Val Cys Ser Ser Cys Pro Val Gly Thr Phe
                85                  90                  95
Thr Arg His Glu Asn Gly Ile Glu Lys Cys His Asp Cys Ser Gln Pro
            100                 105                 110
Cys Pro Trp Pro Met Ile Glu Lys Leu Pro Cys Ala Ala Leu Thr Asp
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115 120 125

Arg Glu Cys Thr Cys Pro Pro Gly Met Phe Gln Ser Asn Ala Thr Cys
130 135 140

Ala Pro His Thr Val Cys Pro Val Gly Trp Gly Val Arg Lys Lys Gly
145 150 155 160

Thr Glu Thr Glu Asp Val Arg Cys Lys Gln Cys Ala Arg Gly Thr Phe
165 170 175

Ser Asp Val Pro Ser Ser Val Met Lys Cys Lys Ala Tyr Thr Asp Cys
180 185 190

Leu Ser Gln Asn Leu Val Val Ile Lys Pro Gly Thr Lys Glu Thr Asp
195 200 205

Asn Val Cys Gly Thr Leu Pro Ser Phe Ser Ser Ser Thr Ser Pro Ser
210 215 220

Pro Gly Thr Ala Ile Phe Pro Arg Pro Glu His Met Glu Thr His Glu
225 230 235 240

Val Pro Ser Ser Thr Tyr Val Pro Lys Gly Met Asn Ser Thr Glu Ser
245 250 255

Asn Ser Ser Ala Ser Val Arg Pro Lys Val Leu Ser Ser Ile Gln Glu
260 265 270

Gly Thr Val Pro Asp Asn Thr Ser Ser Ala Arg Gly Lys Glu Asp Val
275 280 285

Asn Lys Thr Leu Pro Asn Leu Gln Val Val Asn His Gln Gln Gly Pro
290 295 300

His His Arg His Ile Leu Lys Leu Leu Pro Ser Met Glu Ala Thr Gly
305 310 315 320

Gly Glu Lys Ser Ser Thr Pro Ile Lys Gly Pro Lys Arg Gly His Pro
325 330 335

Arg Gln Asn Leu His Lys His Phe Asp Ile Asn Glu His Leu Pro Trp
340 345 350

Met Ile Val Leu Phe Leu Leu Leu Val Leu Val Val Ile Val Val Cys
355 360 365

Ser Ile Arg Lys Ser Ser Arg Thr Leu Lys Lys Gly Pro Arg Gln Asp
370 375 380

Pro Ser Ala Ile Val Glu Lys Ala Gly Leu Lys Lys Ser Met Thr Pro
385 390 395 400

Thr Gln Asn Arg Glu Lys Trp Ile Tyr Tyr Cys Asn Gly His Gly Ile
405 410 415

Asp Ile Leu Lys Leu Val Ala Ala Gln Val Gly Ser Gln Trp Lys Asp
420 425 430

Ile Tyr Gln Phe Leu Cys Asn Ala Ser Glu Arg Glu Val Ala Ala Phe
435 440 445

Ser Asn Gly Tyr Thr Ala Asp His Glu Arg Ala Tyr Ala Ala Leu Gln

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    450                 455                 460
His Trp Thr Ile Arg Gly Pro Glu Ala Ser Leu Ala Gln Leu Ile Ser
465                 470                 475                 480
Ala Leu Arg Gln His Arg Arg Asn Asp Val Val Glu Lys Ile Arg Gly
                485                 490                 495
Leu Met Glu Asp Thr Thr Gln Leu Glu Thr Asp Lys Leu Ala Leu Pro
            500                 505                 510
Met Ser Pro Ser Pro Leu Ser Pro Ser Pro Ile Pro Ser Pro Asn Ala
        515                 520                 525
Lys Leu Glu Asn Ser Ala Leu Leu Thr Val Glu Pro Ser Pro Gln Asp
    530                 535                 540
Lys Asn Lys Gly Phe Phe Val Asp Glu Ser Glu Pro Leu Leu Arg Cys
545                 550                 555                 560
Asp Ser Thr Ser Ser Gly Ser Ser Ala Leu Ser Arg Asn Gly Ser Phe
                565                 570                 575
Ile Thr Lys Glu Lys Lys Asp Thr Val Leu Arg Gln Val Arg Leu Asp
            580                 585                 590
Pro Cys Asp Leu Gln Pro Ile Phe Asp Asp Met Leu His Phe Leu Asn
        595                 600                 605
Pro Glu Glu Leu Arg Val Ile Glu Glu Ile Pro Gln Ala Glu Asp Lys
    610                 615                 620
Leu Asp Arg Leu Phe Glu Ile Ile Gly Val Lys Ser Gln Glu Ala Ser
625                 630                 635                 640
Gln Thr Leu Leu Asp Ser Val Tyr Ser His Leu Pro Asp Leu Leu
                645                 650                 655
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 584 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GCGNCCGCGN NGNGNGCAAG GTGCTGAGCG CCCCTAGNGC CTCCCTTGCC GCCTCCCTCC    60
TCTGCCCGGC CGTAGCAGTG CACATGGGGT GTTGGAGGTA GATGGGCTCC CGGCCGGGAG   120
GCGGCGGTGG ATGCGGCGCT GGGCAGAAGC AGCCGCCGAT TCCAGCTGCC CCGCGCGCCC   180
CGGCCACCTT GCGAGTCCCC GGTTCAGCCA TGGGGACCTC TCCGAGCAGC AGCACCGCCC   240
TCGCCTCCTG CAGCCGCATC GCCCGCCGAG CCACAGCCAC GATGATCGCG GGCTCCCTTC   300
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TCCTGCTTGG ATTCCTTAGC ACCACCACAG CTCAGCCAGA ACAGAAGGCC TCGAATCTCA    360
TTGGCACATA CCGCCATGTT GACCGTGCCA CCGGCCAGGT GCTAACCTGT GACAAGTGTC    420
CAGCAGGAAC CTATGTCTCT GAGCATTGTA CCAACACAAG CCTGCGCGTC TGCAGCAGTT    480
GCCCTGTGGG GACCTTTACC AGGCATGAGA ATGGCATAGA GAAATGCCAT GACTGTAGTC    540
AGCCATGCCC ATGGCCAATG ATTGAGAAAT TACCTTGTGC TGCC                      584
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gly Thr Ser Pro Ser Ser Ser Thr Ala Leu Ala Ser Cys Ser Arg
 1               5                  10                  15
Ile Ala Arg Arg Ala Thr Ala Thr Met Ile Ala Gly Ser Leu Leu Leu
            20                  25                  30
Leu Gly Phe Leu Ser Thr Thr Thr Ala Gln Pro Glu Gln Lys Ala Ser
        35                  40                  45
Asn Leu Ile Gly Thr Tyr Arg His Val Asp Arg Ala Thr Gly Gln Val
    50                  55                  60
Leu Thr Cys Asp Lys Cys Pro Ala Gly Thr Tyr Val Ser Glu His Cys
65                  70                  75                  80
Thr Asn Thr Ser Leu Arg Val Cys Ser Ser Cys Pro Val Gly Thr Phe
                85                  90                  95
Thr Arg His Glu Asn Gly Ile Glu Lys Cys His Asp Cys Ser Gln Pro
            100                 105                 110
Cys Pro Trp Pro Met Ile Glu Lys Leu Pro Cys Ala Ala
        115                 120                 125
```

Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the TR7 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO: 1 encoding the TR7 polypeptide of SEQ ID NO2.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

5. The polynucleotide of claim 1 which is DNA or RNA.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a TR7 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a TR7 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a TR7 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a TR7 polypeptide.

10. A TR7 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the TR7 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of TR7 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or
(b) providing to the subject polynucleotide of claim 1 in a form so as to effect production of said receptor activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of TR7 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or
(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor; and/or
(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TR7 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TR7 polypeptide in the genome of said subject; and/or
(b) analyzing for the presence or amount of the TR7 polypeptide expression in a sample derived from said subject.

16. A method for identifying agonists to TR7 polypeptide of claim 10 comprising:

(a) contacting cells produced by claim 9 with a candidate compound; and
(b) determining whether the candidate compound effects a signal generated by activation of the TR7 polypeptide.

17. An agonist identified by the method of claim 16.

18. The method for identifying antagonists to TR7 polypeptide of claim 10 comprising:

(a) contacting said cell produced by claim 9 with an agonist; and
(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

19. An antagonist identified by the method of claim 18.

20. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a TR7 polypeptide.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 30 2528

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	KWON, B.S. ET AL.: "A newly identified member of the tumor necrosis factor receptor superfamily" J. ALLERG. CLIN. IMMUNOL., vol. 99, no. 1, January 1997, page s467 XP002072602 * abstract 1897 * ---	1-16,18, 20	C12N15/12 C07K14/715 A61K48/00 G01N33/50 C07K16/28
Y	DATABASE EMBL entryname HS073E08B, 27 August 1995 FUJIWARA, T. ET AL.: "unpublished" XP002072603 * abstract * ---	1-16,18, 20	
Y	WO 96 34095 A (HUMAN GENOME SCIENCES) 31 October 1996 * claims * -----	1-16,18, 20	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07K
C12N
A61K
G01N

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

A compound is not sufficienty characterised by a method for its identification (claims 17 and 19).

Place of search	Date of completion of the search	Examiner
MUNICH	24 July 1998	Hermann, R

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)